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(54) **Marek's disease virus vaccine.**

(57) The invention is concerned with the MD18 and MD20 polypeptides of Marek's Disease virus which can be used to vaccinate poultry against MD.

The invention also relates to nucleic acid sequences encoding the MD18 or MD20 polypeptides. Said sequences can be used for the preparation of a subunit or vector vaccine.

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The present invention is concerned with a nucleic acid sequence encoding a Marek's Disease virus polypeptide, a recombinant nucleic acid molecule comprising such a nucleic acid sequence, a vector virus comprising said nucleic acid sequence, a host cell transformed with such a nucleic acid sequence, a Marek's Disease virus polypeptide and antibodies reactive therewith, as well as a vaccine against Marek's Disease.

Marek's Disease (MD) is a malignant, lymphoproliferative disorder of domestic fowl caused by a herpesvirus: Marek's Disease Virus (MDV). MD is ubiquitous, occurring in poultry-producing countries throughout the world. Chickens raised under intensive production systems will inevitably suffer losses from MD. MD affects chickens from about 6 weeks of age, occurring most frequently between ages of 12 and 24 weeks.

Three forms of MD are recognized clinically, classical MD, acute MD and transient paralysis.

Classical MD is characterized by peripheral nerve enlargement caused by lymphoid infiltration and demyelination, and paralysis is the dominant clinical sign. Mortality is variable but normally under 10-15 per cent.

In the acute form there are multiple and diffuse lymphomatous tumours in the visceral organs. Mortality from this form of MD is usually higher than from the classical form. An incidence of 10-30 per cent is common in unvaccinated flocks and outbreaks involving up to 70% of the flock may be encountered. The pathological lesions in both classical and acute MD are essentially similar, involving the proliferation and infiltration of malignantly transformed T-lymphoblasts into normal tissues, peripheral nerves in the case of the classical form and visceral organs in the case of the acute form.

Furthermore, the MDV has been shown to be responsible for encephalitis of young chickens characterized by sudden paralysis.

Serological classification of MD related viruses yielded three serotypes:

Type I : naturally occurring virulent strains of Marek's disease virus which are pathogenic and tumorigenic to chickens, and attenuated nonpathogenic strains derived therefrom

Type II : naturally occurring nonpathogenic strains of Marek's disease virus; and

Type III : herpesvirus of turkeys ("HVT"), which is nonpathogenic to chickens.

Serial passage of pathogenic strains of MDV serotype I was found to result in loss of pathogenicity and oncogenicity, but not of immunogenicity. Attenuated strains derived from HPRS-16 and CVI-988 strains have been applied as vaccines. SB-I and HN-I MDV strains (serotype 2) have also been shown to be useful in vaccination. HUT, first isolated from turkeys, is apathogenic in turkeys and domestic fowls, antigenically related to serotype 1 and 2 MD viruses and extensively used as a vaccine against MD.

There are no methods of treatment of MD and control is based on management methods which isolate growing chickens from sources of infection, the use of genetically resistant stock, and vaccination. However, management procedures are normally not cost-effective and the progress has been disappointing with respect to the selection of poultry stock with increased genetically controlled resistance. Nowadays, control of MD is almost entirely based on vaccination.

Current vaccines comprise chemically inactivated virus vaccines or modified live-virus vaccines. However, inactivated vaccines require additional immunizations, disadvantageously contain adjuvants, are expensive to produce and are laborious to administer. Further, some infectious virus particles may survive the inactivation process and may cause disease after administration to the animal.

In general, attenuated live virus vaccines are preferred because they evoke an immune response often based both humoral and cellular reactions. Up to now, such vaccines based on MDV serotype I strains can only be prepared by serial passage of virulent strains in tissue culture. However, because of this treatment uncontrolled mutations are introduced into the viral genome, resulting in a population of virus particles heterogeneous with regard to virulence and immunizing properties. Overattenuation during passage in cell culture can also be a problem with these vaccines. One must achieve a delicate balance between ensuring that the vaccine is not virulent while making certain that it is still protective. In addition it is well known that such traditional attenuated live virus vaccines can revert to virulence resulting in disease outbreaks in inoculated animals and the possible spread of the pathogen to other animals. The occurrence of very virulent field strains of MD virus against which live HVT vaccines provided poor protection have now been isolated and are responsible for excessive losses in various parts of the world. Bivalent vaccines consisting of serotype 2 and serotype 3 strains are reasonably effective against very virulent field isolates in some cases. Multivalent vaccines containing serotype 1 antigens should be even more effective at eliciting immunity against these very virulent strains.

Improved vaccines might be constructed based on recombinant DNA technology. These vaccines would only contain the necessary and relevant MDV immunogenic material which is capable of eliciting a protective immune response against the MDV pathogens, or the genetic information encoding said material, and would not display above-mentioned disadvantages of the live or inactivated vaccines.

According to the present invention a nucleic acid sequence encoding a MDV polypeptide is provided which can be applied for the preparation of a vaccine for the immunization of poultry against MD.

"Nucleic acid sequence" as used herein refers to a polymeric form of nucleotides of any length, both to ribonucleic acid sequences and to deoxy-ribonucleic acid sequences. In principle, this term refers to the primary structure of the molecule. Thus, this term includes double and single stranded DNA, as well as double and single stranded RNA, and modifications thereof.

In general, the term "polypeptide" refers to a molecular chain of amino acids with a biological activity and does not refer to a specific length of the product. If required the polypeptide can be modified in vivo or in vitro, for example by glycosylation, amidation, carboxylation or phosphorylation; thus inter alia, peptides, oligopeptides and proteins are included.

According to the invention a nucleic acid sequence containing a gene encoding the MDV polypeptide MD18 or MD20, respectively, have been isolated and characterized and were found to be recognized by the immune system of the host. The genes encoding said polypeptides were identified by screening bacteriophage expression libraries made in the lambda gt11 vector, with polyvalent sera from chickens infected with a virulent MD virus strain.

The gene encoding the MD18 polypeptide maps to the unique long (U_L) region of the MDV genome and encodes a polypeptide of about 663 amino acids in length. The amino acid sequence of the polypeptide encoded by the MD18 gene is shown in SEQ ID NO: 2.

The gene encoding the MD20 polypeptide also maps to the U_L region of the MDV genome and encodes a polypeptide of about 1074 amino acids in length. The amino acid sequence of the polypeptide encoded by the MD20 gene is shown in SEQ ID NO: 4.

More particularly, this invention provides a nucleic acid sequence that encodes the MD18 polypeptide or MD20 polypeptide having an amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4, respectively.

Also included within the scope of the present invention are nucleic acid sequences encoding a functional equivalent of said MD18 or MD20 polypeptide having corresponding immunological characteristics.

It will be understood that for the particular MD18 or MD20 polypeptide embraced herein, derived from the serotype 1 GA strain, natural variations can exist between individual viruses or strains of MDV of type 1. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions from which can be expected that they do not essentially alter biological and immunological activities have been described. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the similarity between homologous polypeptides. Nucleic acid sequences encoding such functional equivalents are included within the scope of this invention. Moreover, the potential exist to use recombinant DNA technology for the preparation of nucleic acid sequences encoding these various functional equivalents.

Preferably, nucleic acid sequences according to the invention may be derived from available isolates of MDV of serotype 1, strains such as GA, JM, HPRS-16, Conn A, RB-IB CVI-988 or Md 11, the GA strain being the most preferred strain.

In addition nucleic acid sequences encoding the MD18 polypeptide or MD20 polypeptide or variations thereof as mentioned above may also be derived from MDV strains belonging to serotype 2 or serotype 3, e.g. HN, HPRS-24, SB-1 or FC126.

The information provided in SEQ ID NO: 1-4 allows a person skilled in the art to isolate and identify the nucleic acid sequences encoding the variant functional equivalent polypeptides mentioned-above having corresponding immunological characteristics with the MD18 or MD20 polypeptide disclosed herein. The generally applied blotting and hybridization techniques can be used for that purpose (Experiments in Molecular Biology, ed. R.J. Slater, Clifton, U.S.A., 1986; Singer-Sam, J. et al., Proc. Natl. Acad. Sci. 80, 802-806, 1983; Maniatis, T. et al., in Molecular Cloning, A laboratory Manual, Cold Spring Harbor Laboratory Press, U.S.A., 1989). For example, restriction enzyme digested DNA fragments derived from a specific MDV strain is electrophoresed and transferred, or "blotted" thereafter onto a piece of nitrocellulose filter. It is now

possible to identify the nucleic sequences encoding the functional equivalent polypeptides on the filter by hybridization to a defined labelled DNA or "probe" back translated from the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4, under specific conditions of salt concentration and temperature that allow hybridization of the probe to any homologous DNA sequences present on the filter. After washing the filter, hybridized material may be detected by autoradiography. From an agarose gel with starting DNA that was not blotted, DNA can now be obtained that encodes a polypeptide functionally equivalent to a polypeptide disclosed in SEQ ID NO: 2 or 4.

In another way, DNA obtained from a specific MDV strain may be cloned into a λgt11 phage and expressed into a bacterial host. Recombinant phages can then be screened with polyclonal serum raised against the purified MD18 or MD20 polypeptide, determining the corresponding immunological characteristic of the variant polypeptide. Above-mentioned procedure is outlined in detail in Example 1. The production of the polyclonal serum elicited against MD18 or MD20 is described below.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in another codon but still coding for the same amino acid, e.g. the codon for the amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of a polypeptide with the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4 use can be made of a derivate nucleic acid sequence (functional equivalent) with such an alternative codon composition different from the nucleic acid sequence shown in said SEQ ID's.

A preferred nucleic acid sequence according to the invention is characterized in that said sequence contains the deoxyribonucleic acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 3.

Furthermore, also fragments of the nucleic acid sequences encoding the MD18 or MD20 polypeptide or functional equivalents thereof as mentioned above are included in the present invention.

The term "fragment" as used herein means a DNA or amino acid sequence comprising a subsequence of one of the nucleic acid sequences or polypeptides of the invention. Said fragment is or encodes a polypeptide having one or more immunoreactive and/or antigenic determinants of a MDV polypeptide, i.e. has one or more epitopes which are capable of eliciting an immune response in a chicken and/or is capable of specifically binding to a complementary antibody. Methods for determining usable polypeptide fragments are outlined below. Fragments can inter alia be produced by enzymatic cleavage of precursor molecules, using restriction endonucleases for the DNA and proteases for the polypeptides. Other methods include chemical synthesis of the fragments or the expression of polypeptide fragments by DNA fragments introduced in a suitable host cell environment.

All modifications resulting in such functional equivalents of the MD18 or MD20 polypeptide are included within the scope of the present invention for as long as the immunological characteristics of the MD18 or MD20 polypeptide remain unaffected in essence.

A nucleic acid sequence according to the present invention can be ligated to various replication effecting DNA sequences with which it is not associated or linked in nature, optionally containing portions of DNA encoding fusion protein sequences such as β-galactosidase, resulting in a so called recombinant nucleic acid molecule which can be used for the transformation of a suitable host. Such hybrid DNA molecules, are preferably derived from, for example plasmids, or from nucleic acid sequences present in bacteriophages, cosmids or viruses. Specific vectors which can be used to clone nucleic acid sequences according to the invention are known in the art and include inter alia plasmid vectors such as pBR322, the various pUC, pGEM and Bluescript plasmids, bacteriophages, e.g. λgt-Wes-λ B, Charon 28 and the M13 derived phages or viral vectors such as SV40, adenovirus or polyoma virus (see also Rodriquez, R.L. and D.T. Denhardt, ed., Vectors: A survey of molecular cloning vectors and their uses, Butterworths, 1988; Lenstra, J.A. et al., Arch. Virol. 110, 1-24, 1990). The methods to be used for the construction of a recombinant nucleic acid molecule according to the invention are known to those of ordinary skill in the art and are inter alia set forth in Maniatis, T. et al. (ibid, 1989). For example, the insertion of the nucleic acid sequence according to the invention into a cloning vector can easily be achieved by ligation with an enzyme such as T_4 DNA ligase when both the genes and the desired cloning vehicle have been cut with the same restriction enzyme(s) as complementary DNA termini are thereby produced.

Alternatively, it may be necessary to modify the restriction sites that are produced into blunt ends either by digesting the single-stranded DNA or by filling in the recessive termini with an appropriate DNA polymerase. Subsequently, blunt end ligation with an enzyme such as T4 DNA ligase may be carried out.

If desired, any restriction site may be produced by ligating linkers onto the DNA termini. Such linkers may comprise specific oligonucleotide sequences that encode restriction site sequences. The restriction enzyme cleaved vector and nucleic acid sequence may also be modified by homopolymeric tailing.

"Transformation", as used herein, refers to the introduction of a heterologous nucleic acid sequence into a host cell, irrespective of the method used, for example direct uptake or transduction. The heterologous nucleic acid sequence may be maintained through autonomous replication or alternatively, may be integrated into the host genome. If desired, the recombinant DNA molecules are provided with appropriate control sequences compatible with the designated host which can regulate the expression of the inserted nucleic acid sequence.

The recombinant nucleic acid molecule according to the invention preferably contains one or more marker activities that may be used to select for desired transformants, such as ampicillin and tetracycline resistance in pBR322, ampicillin resistance and β-galactosidase activity in pUC8.

A suitable host cell is a cell which can be transformed by a nucleic acid sequence encoding a polypeptide or by a vector virus or a recombinant nucleic acid molecule comprising such a nucleic acid sequence and which can if desired be used to express said polypeptide encoded by said nucleic acid sequence. The host cell can be of procaryotic origin, e.g. bacteria such as Escherichia coli, Bacillus subtilis and Pseudomonas species; or of eucaryotic origin such as yeast, e.g. Saccharomyces cerevisiae or higher eucaryotic cells such as insect, plant or mammalian cells, including HeLa cells and Chinese hamster ovary (CHO) cells. Insect cells include the Sf9 or Sf21 cell line of Spodoptera frugiperda (Luckow et al., Biotechnology 6, 47-55, 1989). Information with respect to the cloning and expression of the nucleic acid sequence of the present invention in eucaryotic cloning systems can be found in Esser, K. et al. (Plasmids of Eukaryotes, Springer-Verlag, 1986).

In general, prokaryotes are preferred for cloning and manipulation of DNA sequences and for constructing the vectors useful in the invention. For example E. coli K12 is particularly useful. Other microbial strains which may be used include E. coli strains such as DH5α, JM101 or HB101.

For expression, nucleic acid sequences of the present invention are operably linked to expression control sequences. Such control sequences may comprise promoters, enhancers, operators, inducers, ribosome binding sites etc.

When the host cells are bacteria, illustrative useful expression control sequences include the Trp promoter and operator (Goeddel, et al., Nucl. Acids Res. 8, 4057, 1980); the lac promoter and operator (Chang, et al., Nature 275, 615, 1978); the outer membrane protein (OMP) promoter (Nakamura, K. and Inouge, M., EMBO J. 1, 771-775, 1982); the bacteriophage λ promoters and operators (Remaut, E. et al., Nucl. Acids Res. 11, 4677-4688, 1983); the α-amylase (B. subtilis) promoter and operator, termination sequence and other expression enhancement and control sequences compatible with the selected host cell. When the host cell is yeast, illustrative useful expression control sequences include, e.g., α-mating factor. For insect cells the polyhedrin or p10 promoters of baculoviruses can be used (Smith, G.E. et al., Mol. Cell. Biol. 3, 2156-65, 1983). When the host cell is of mammalian origin illustrative useful expression control sequences include, e.g., the SV-40 promoter (Berman, P.W. et al., Science 222, 524-527, 1983) or, e.g. the metallothionein promoter (Brinster, R.L., Nature 296, 39-42, 1982) or a heat shock promoter (Voellmy et al., Proc. Natl. Acad. Sci. USA 82, 4949-53, 1985). Alternatively, also expression control sequences present in MDV, in particular those regulating the expression of MD18 or MD20 may be applied. For maximizing gene expression, see also Roberts and Lauer (Methods in Enzymology 68, 473, 1979).

The present invention also comprises a polypeptide displaying MDV immunological characteristics containing at least part of the MD18 or MD20 amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4, respectively, or derivatives thereof, essentially free from the whole virus or other protein with which it is ordinarily associated.

It will be understood that derivatives of said amino acid sequences displaying the same immunological properties in essence, i.e. immunological equivalents, are also within the scope of the present invention.

Immunological equivalents of the MD18 or MD20 polypeptide disclosed herein are the corresponding polypeptides present in viruses of other strains of MD serotype 1 or in viruses of strains belonging to serotype 2 or 3. Said equivalents can be produced through the expression of the genes encoding said equivalents, the genes being identified and isolated making use of the information provided herein as described above.

In addition a polypeptide comprising a fragment of the MD18 or MD20 polypeptide or functional equivalent thereof, which can be used for immunization of poultry against MD is included in the present invention. Various methods are known for detecting such usable polypeptide fragments within a known amino acid sequence.

Suitable immunochemically active polypeptide fragments of a polypeptide according to the invention containing (an) epitope(s) can be found by means of the method described in Patent Application WO 86/06487, Geysen, H.M. et al. (Prod. Natl. Acad. Sci. 81, 3998-4002, 1984), Geysen, H.M. et al. (J. Immunol.

Meth. 102, 259-274, 1987) based on the so-called pep-scan method, wherein a series of partially overlapping polypeptides corresponding with partial sequences of the complete polypeptide under consideration, are synthesized and their reactivity with antibodies is investigated.

In addition, a number of regions of the polypeptide, with the stated amino acid sequence, can be designated epitopes on the basis of theoretical considerations and structural agreement with epitopes which are now known.

The determination of these regions was based on a combination of the hydrophilicity criteria according to Hopp and Woods (Proc. Natl. Acad. Sci. 78, 3824-3828, 1981) and the secondary structure aspects according to Chou and Fasman (Advances in Enzymology 47, 45-148, 1987).

T-cell epitopes which may be necessary can likewise be derived on theoretical grounds with the aid of Berzofsky's amphiphilicity criterion (Science 235, 1059-62, 1987).

Such epitopes can also be generated experimentally by limited exposure of the polypeptide to the proteolytic activity of Cathepsin D. Cleavage of a protein antigen by this enzyme specifically recognizes amino acid sequence patterns which are also found within the NH_2-terminal residues of peptides recognized by the major histocompability complex on the surface of antigen-presenting cells (v. Noort, J.M. and v.d. Drift, A.C.M., J. Biol. Chem. 264, 14159, 1989).

In another embodiment of the invention a polypeptide having an amino acid sequence encoded by a nucleic acid sequence mentioned above is used.

Immunization of poultry against MDV infection can, for example be achieved by administering to the animals a polypeptide according to the invention in an immunologically relevant context as a so-called subunit vaccine. The subunit vaccine according to the invention may comprise a polypeptide in a pure form, optionally in the presence of a pharmaceutically acceptable carrier. The polypeptide can optionally be covalently bonded to a non-related protein, which, for example can be of advantage in the purification of the fusion product. Examples are β-galactosidase, protein A, prochymosine, blood clotting factor Xa, etc.

In some cases the ability to raise neutralizing antibodies against these polypeptides per se may be low. Small fragments are preferably conjugated to carrier molecules in order to increase their immunogenicity. Suitable carriers for this purpose are macromolecules, such as natural polymers (proteins like keyhole limpet hemocyanin, albumin, toxins), synthetic polymers like polyamino acids (polylysine, polyalanine), or micelles of amphiphilic compounds like saponins. Alternatively these fragments may be provided as polymers thereof, preferably linear polymers.

Polypeptides to be used in such subunit vaccines can be prepared by methods known in the art, e.g. by isolating said polypeptides from MDV, by recombinant DNA techniques or by chemical synthesis.

If required the polypeptides according to the invention to be used in a vaccine can be modified in vitro or in vivo, for example by glycosylation, amidation, carboxylation or phosphorylation.

An alternative to subunit vaccines are live vector vaccines. A nucleic acid sequence according to the invention is introduced by recombinant DNA techniques into a micro-organism (e.g. a bacterium or virus) in such a way that the recombinant micro-organism is still able to replicate thereby expressing a polypeptide coded by the inserted nucleic acid sequence.

For example the technique of in vivo homologous recombination can be used to introduce a heterologous nucleic acid sequence, e.g. a nucleic acid sequence according to the invention into the genome of the vector micro-organism.

First, a DNA fragment corresponding with an insertion region of the vector genome, i.e. a region which can be used for the incorporation of a heterologous sequence without disrupting essential functions of the vector such as those necessary for infection or replication, is inserted into a cloning vector according to standard recDNA techniques. Insertion-regions have been reported for a large number of micro-organisms (e.g. EP 80,806, EP 110,385, EP 83,286, EP 314,569, WO 88/02022 and WO 88/07088).

Second, if desired, a deletion can be introduced into the insertion region present in the recombinant DNA molecule obtained from the first step. This can be achieved for example by appropriate exonuclease III digestion or restriction enzyme treatment of the recombinant DNA molecule from the first step.

Third, the heterologous nucleic acid sequence is inserted into the insertion-region present in the recombinant DNA molecule of the first step or in place of the DNA deleted from said recombinant DNA molecule. The insertion-region DNA sequence should be of appropriate length as to allow homologous recombination with the vector genome to occur. Thereafter, suitable cells can be transformed with vector genomic DNA in the presence of the recombinant DNA molecule containing the insertion flanked by appropriate vector DNA sequences whereby recombination occurs between the corresponding regions in the recombinant DNA molecule and the vector genome. Recombinant vector progeny can now be produced in cell culture and can be selected for example genotypically or phenotypically, e.g. by hybridization,

detecting enzyme activity encoded by a gene co-integrated along with the heterologous nucleic acid sequence, or detecting the antigenic heterologous polypeptide expressed by the recombinant vector immunologically.

Next, this recombinant micro-organism can be administered to the host animal for immunization whereafter it is maintained and optionally replicates in the body of the inoculated animal, expressing in vivo a polypeptide coded for by the nucleic acid sequence according to the invention inserted in a vector organism and resulting in the stimulation of the immune system of the inoculated animal.

Suitable vectors for the incorporation of a nucleic acid sequence according to the invention can be derived from viruses such as (avian) pox viruses, e.g. vaccinia virus or fowl pox virus (EP 314,569 and WO 88/02022), herpes viruses such as HVT (WO 88/07088), adeno virus or influenza virus, or bacteria such as E. coli or specific Salmonella species. With recombinant micro-organisms of this type, the polypeptide synthesized in the host cell can be exposed as a surface antigen. In this context fusion of the said polypeptide with OMP proteins, or pilus proteins of for example E. coli or with synthetic provision of signal and anchor sequences which are recognized by the organism are conceivable. It is also possible that the said immunogenic polypeptide, if desired as part of a larger whole, is released inside the animal to be immunized. In all of these cases it is also possible that one or more immunogenic products will be expressed, generating protection against various pathogens and/or against various antigens of a given pathogen.

A vaccine according to the invention can be prepared by culturing a host cell infected with a vector virus comprising a nucleic acid sequence according to the invention, whereafter virus containing cells and/or vector viruses grown in the cells can be collected, optionally in a pure form, and formed to a vaccine optionally in a lyophilized form.

Above-mentioned host cells comprising a nucleic acid sequence according to the invention can also be cultured under conditions which are favourable for the expression of a polypeptide coded by said nucleic acid sequence. Vaccines may be prepared using samples of the crude culture, host cell lysates or host cell extracts, although in another embodiment more purified polypeptides according to the invention are formed to a vaccine, depending on its intended use. In order to purify the polypeptides produced, host cells containing a nucleic acid sequence according to the invention are cultured in an adequate volume and the polypeptides produced are isolated from such cells or from the medium if the protein is secreted. Polypeptides secreted into the medium can be isolated and purified by standard techniques, e.g. salt fractionation, centrifugation, ultrafiltration, chromatography, gel filtration or immuno affinity chromatography, whereas intracellular polypeptides can be isolated by first collecting said cells, disrupting the cells, for example by sonication or by other mechanically disruptive means such as French press followed by separation of the polypeptides from the other intracellular components and forming the polypeptides to a vaccine. Cell disruption could also be accomplished by chemical (e.g. EDTA treatment) or enzymatic means such as lysozyme digestion.

Antibodies or antiserum directed against a polypeptide according to the invention have potential use in passive immunotherapy, diagnostic immunoassays and generation of anti-idiotype antibodies.

The MDV polypeptides MD18 or MD20 as described above can be used to produce antibodies, both polyclonal, monospecific and monoclonal. If polyclonal antibodies are desired, techniques for producing and processing polyclonal sera are known in the art (e.g. Mayer and Walter, eds, Immunochemical Methods in Cell and Molecular Biology, Academic Press, London, 1987). In short, a selected mammal, e.g. rabbit is given (multiple) injections with one of the above-mentioned immunogens, about 20 µg to about 80 µg of protein per immunization. Immunizations are given with an acceptable adjuvant, generally equal volumes of immunogen and adjuvant. Acceptable adjuvants include Freund's complete, Freund's incomplete, alum-precipitate or water-in-oil emulsions, with Freund's complete adjuvant being preferred for the initial immunization. Freund's incomplete adjuvant is preferred for all booster immunizations. The initial immunization consists of the administration of about 1 ml of emulsion at multiple subcutaneous sites on the backs of the rabbits. Booster immunizations utilizing an equal volume of immunogen are given at about one month intervals and are continued until adequate levels of antibodies are present in an individual rabbits serum. Blood is collected and serum isolated by methods known in the art.

Monospecific antibodies to each of the immunogens are prepared by immunizing rabbits as described above with the purified proteins and thereafter affinity purified from polyspecific antisera by a modification of the method of Hall et al. (Nature 311, 379-387 1984). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogeneous binding characteristics for the relevant antigen. Homogeneous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope.

Monoclonal antibody reactive against each of the MDV immunogens can be prepared by immunizing inbred mice, preferably Balb/c with the appropriate protein. The mice are immunized intraperitoneally with about 100 ng to about 10 µg immunogen per 0.5 ml in an equal volume of a suitable adjuvant. Such acceptable adjuvants include Freund's complete, Freund's incomplete, alum-precipitate and water-in-oil emulsions. The mice are given intravenous booster immunizations of an equal amount of the immunogen without adjuvant at about days 14, 21 and 63 post primary immunization. At about day three after the final booster immunization, individual mice are serologically tested for anti-immunogen antibodies. Spleen cells from antibody producing mice are isolated and fused with murine myeloma cells, such as SP-2/0 or the like, by techniques known in the art (Kohler and Milstein, Nature 256; 495-497, 1975). Hybridoma cells are selected by growth in appropriate cell culture medium such as Dulbecco's modified Eagle's medium (DMEM) containing hypoxanthine, thymidine and aminopterin in an antibody producing hybridomas are cloned, preferably using the soft agar technique of MacPherson (Soft Agar Techniques, Tissue Culture Methods and Applications, Kruse and Paterson, eds., Academic Press, 276, 1973), Discrete colonies are transferred into individual wells of culture plates for cultivation in an appropriate culture medium. Antibody producing cells are identified by screening with the appropriate immunogen. Immunogen positive hybridoma cells are maintained by techniques known in the art. Specific monoclonal antibodies are produced by cultivating the hybridomas in vitro or preparing ascites fluid in mice following hybridoma injection by procedures known in the art.

Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the pathogen against which protection is desired and can be used as an immunogen in a vaccine (Dreesman et al., J. Infect. Disease 151, 761, 1985). Techniques for raising anti-idiotype antibodies are known in the art (MacNamara et al., Science 226, 1325, 1984).

The vaccine according to the invention can be administered in a conventional active immunization scheme: single or repeated administration in a manner compatible with the dosage formulation and in such amount as will be prophylactically and/or therapeutically effective and immunogenic. The administration of the vaccine can be done, e.g. intradermally, subcutaneously, intramusculary, intravenously or intranasally.

Additionally, the vaccine may also contain an aqueous medium or a water-containing suspension, often mixed with other constituents, e.g. in order to increase the activity and/or shelf life. These constituents may be salts, pH buffers, stabilizers (such as skimmed milk or casein hydrolysate), emulsifiers, adjuvants to improve the immune response (e.g. oils, muramyl dipeptide, aluminium-hydroxide, tocol derivatives, saponin, polyanions and amphipatic substances) and preservatives.

It is clear that a vaccine according to the invention may also contain immunogens related to other pathogens of poultry or may contain nucleic acid sequences encoding these immunogens, like antigens of Infectious Bronchitis Virus, Newcastle Disease virus Infectious Bursal Disease virus of Marek's Disease Virus different from those disclosed herein, to produce a multivalent vaccine.

The invention also relates to an "immunochemical reagent", which reagent comprises at least one of the polypeptides according to the invention or an antigenic fragment thereof.

The term "immunochemical reagent" signifies that the polypeptides according to the invention have been bound to a suitable support or have been provided with a labelling substance.

The supports which can be used are, for example, the inner wall of a microtest well or a cuvette, a tube or capillary, a membrane, filter, test strip or the surface of a particle such as, for example, a latex particle, an erythrocyte, a dye sol, a metal sol or metal compound as sol particle.

Labelling substances which can be used are, inter alia, a radioactive isotope, a fluorescent compound, an enzyme, a dye sol, metal sol or metal compound as sol particle.

A nucleic acid sequence according to the invention can also be used to design specific probes for hybridization experiments for the detection of MDV related nucleic acids in any kind of tissue.

The present invention also provides a test kit comprising said nucleic acid sequence useful for the diagnosis of MDV infection.

The invention also relates to a test kit to be used in an immuno-assay, this test kit containing at least one immunochemical reagent according to the invention.

The immunochemical reaction which takes place using this test kit is preferably a sandwich reaction, an agglutination reaction, a competition reaction or an inhibition reaction.

For carrying out a sandwich reaction, the test kit can consist, for example, of a polypeptide according to the invention bonded to a solid support, for example the inner wall of a microtest well, and either a labelled polypeptide according to the invention or a labelled anti-antibody.

Example 1

Screening of bacterial expression libraries with convalescent chicken serum

The vector used for establishment of the library has been λgt11 (Young, R.A. and Davis, R.W. Proc. Natl. Acad. Sci 80, 1194-1198, 1983). The genome of this expression vector contains a functional LacZ gene encoding the enzyme β-galactosidase with a unique EcoRI restriction site near the carboxy terminus. Insertion of DNA fragments from the MDV genome at this site, potentially results in the expression of a protein consisting of a MDV specific polypeptide fused to a major part of the β-galactosidase. Libraries representing a complex range of DNA fragments can be screened at high density for recombinant clones producing a fusion protein which is recognized by an antibody probe such as the serum from MDV infected birds. The library for these experiments was made using the DNA of a pool of BamHI plasmid clones which represented about 90% of the viral genome of MDV strain GA (Fukuchi et al., J. Virol. 51, 102, 1984). DNA from these plasmids was digested with BamHI, fragmented by sonication and size-selected by centrifugation on sucrose gradients. Fragments with a size between 0.5 and 4.0 kb were isolated, tailed with dG-residues and inserted by means of a synthetic adaptor into the unique EcoRI-site of the λgt10 vector (Le Bouc et al., FEBS lett. 196, 108, 1986; Huynh et al., in: Cloning Techniques, A Practical Approach, ed. Glover, D., 49-78, 1985) resulting in a library with an effective size of 5×10^4 pfu. Phages were amplified and purified on CsCl-gradients, DNA was extracted and inserts were recovered by restriction with EcoRI. Finally, these inserts were ligated into the EcoRI-site of the λgt11 vector and recombinant phages were screened with chicken serum against MDV. This convalescent serum was obtained by infecting single-comb white leghorns (SPAFAS) with a virulent passage of the GA strain from MDV obtained from Dr. Calnek (Cornell University, NY, USA). Serum was collected over a 12 weeks period and samples were tested individually by indirect fluorescence for binding to MDV plaques in tissue cultures of chicken embryo fibroblasts (CEF).

Six of the sera were selected based on titre and specificity. A mixture of these samples was used in a 1:100 dilution to screen the λgt11 library on nitrocellulose filters according to Young, R.A. et al. (Proc. Natl. Acad. Sci. U.S.A. 82, 2583, 1985). The second antibody for incubation of the filters was an alkaline phosphatase conjugated rabbit-anti-chicken serum (Sigma, St. Louis, USA) and positive signals were developed by the nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate colour reaction (McGadey, Histochemie 23, 180, 1970). Series of candidates were selected and recombinants were plaque-purified until homogeneity. From four of these, the DNA insert in the λgt11 recombinant was recovered as a EcoRI fragment and transferred to the plasmid vector pUC8 (Vieira, J. and Messing, J., Gene 19, 259, 1982) or pGEM3Z (Promega, Madison, USA). The resulting constructs were designated as pMD18, pMD20 and pMD21.

Example 2

Cloning of the MD18 gene and structural analysis of the antigen MD18

The gene encoding the complete amino acid sequence of antigen MD18 was isolated by screening a λEMBL3 library representing the genome of MDV strain GA. The DNA used for establishment of this library was prepared by infecting chicken embryo fibroblasts (CEF) with a tissue-culture adapted passage of MD strain GA provided by Dr. Nonoyama, Showa Univ., St. Petersburg, U.S.A. Cultures were incubated until 90% cytophatic effect (CPE) had developed and total DNA was prepared by proteinase K digestion and phenol/chloroform extraction. DNA was partially digested with Sau 3A (Promega, Madison, USA) and the size fraction of about 20 kb was isolated after separation in a 0,8% agarose gel. DNA fragments were ligated with BamHI/EcoRI digested λEMBL3 DNA (Promega., Madison, USA), packaged in vitro and plated on E.coli strain LE392. Screening of this library (Maniatis, T. et al., 1989, ibid) with the insert from pMD18 resulted among others in the isolation of λGA12 containing a 21 kb DNA mapped by restriction analysis to a region of the MDV viral genome about halfway the U_L structural element. Within this 21 kb of DNA, the position of the sequence hybridizing with pMD18 was defined to a 3.8 kb BamHI fragment which was subcloned as such in both orientations using the vector pGEM3Z and resulting in the plasmids pMD41 and pMD42 respectively. Nucleotide sequence analysis in both orientations of the DNA, was performed on progressively deleted subclones generated with the enzyme exonuclease III as described by Henikoff, S. (Gene 28, 357, 1984). After assemblage of all sequence data and translation of the sequence in the region

of interest, a primary structure was deduced for the antigen originally identified in the immuno-screening by means of the convalescent chicken serum. The complete amino acid sequence of this antigen designated MD18, is presented in SEQ ID NO: 2.

Example 3

Cloning of the MD20 gene and structural analysis of the antigen MD20.

Analysis directly on the insert of pMD20 and pMD21 revealed a partially overlapping nucleotide sequence suggesting that both candidates were representing the same antigen.

The gene encoding the complete amino acid sequence of this antigen was isolated by screening a λEMBL3 library representing the genome of MDV strain GA. The DNA used for establishment of this library was prepared by infecting chicken embryo fibroblasts (CEF) with a tissue-culture adapted passage of MD strain GA provided by Dr. Nonoyama, Showa Univ., St. Petersburg, U.S.A. Cultures were incubated until 90% cytophatic effect (CPE) had developed and total DNA was prepared by proteinase K digestion and phenol/chloroform extraction. DNA was partially digested with Sau 3A (Promega, Madison, USA) and the size fraction of about 20 kb was isolated after separation in a 0,8% agarose gel. DNA fragments were ligated with BamHI/EcoRI digested λEMBL3 DNA (Promega., Madison, USA), packaged in vitro and plated on E.coli strain LE392. Screening of this library (Maniatis, T. et al., 1982, ibid) with the insert from pMD21 resulted in the isolation of clone λGA09. This clone contained a 17 kb DNA insert that is located near the junction of the U_L and IR_L in the MDV viral genome. Referring to the restriction map as published by Fukuchi et al. (J. Virol. 51, 102, 1984), the 17 kb insert included the region in between the S and I_2 BamHI fragments. Restriction mapping on the DNA from λGA09 and hybridization with the insert from pMD21 identified the position of the gene in a 7.5 kb SalI fragment which was subcloned as such in pGEM3Z to result in pMD26. Nucleotide sequence analysis was performed on subclones generated by both the exonuclease III treatment (Henikoff, S., Gene 28, 257, 1984) and the use of convenient restriction sites. The final sequence obtained after assembling all data from the reactions done in both orientations, was translated into the complete amino acid sequence of the antigen denominated MD20 and is shown in SEQ ID NO: 4.

Example 4

Insertion of the genes encoding MDV antigens MD18 and MD20 into the viral genome of herpes virus of turkey (HVT).

Based on the genome structure of HVT as published by Igarashi, T. et al. (Virology 157, 351, 1987) a region in the unique-short sequence element (Us) of the virus was selected for the insertion of foreign genes. The corresponding DNA fragment was screened from a λEMBL3 library constructed by partially digesting total DNA from HVT infected CEF following a procedure used previously for MDV strain GA. The insert of one of the λ-isolates, characterized by the absence of any BamHI restriction site, was denominated λHVT04 and analyzed in detail by physical mapping (Figure 1). The sequence present in the 17.5 kb inserted fragment represented a major part of the Us region including part of the inverted repeat structure (Igarashi, T. et al., 1987, ibid). One of the 1.2 kb XhoI restriction fragments from λHVT04 was subcloned in pGEM3Z digested with Sal I resulting in plasmid pMD07 which contained a unique BglII site available for insertion of DNA fragments. The gene encoding antigen MD18 or MD20 was assembled from pMD41 and pMD26 respectively by removal of the excess of nucleotide sequences preceding the ATG-initiator and the creation of convenient restriction sites such as Sal I or XhoI flanking the coding region. For pMD41 this resulted in pMD46, and pMD26 gave pMD47, both restriction maps being presented in Figure 2.

A strong promoter which could direct the expression of foreign genes after their insertion into the genome of the HVT virus was selected from the LTR sequence of Rous Sarcoma Virus (RSV). The promoter has been mapped on a 580 bp NdeI/HindIII restriction fragment from pRSVcat (Gorman et al., Proc. Natl. Acad. Sci. 79, 6777, 1982) and was inserted between the HindIII and PstI sites of pGEM3Z (Promega) by means of double stranded synthetic linkers on both sides of the fragment. The connection between the HindIII site from the vector pGEM3Z and the NdeI site of the RSV fragment carrying the LTR-promoter was made with a 30 bp linker containing cohesive ends compatible with HindIII on one and NdeI on the other site. However, after ligation both restriction sites are not restored due to deliberate modifications in the outer nucleotides of the six basepair recognition sequence. In addition to the removal of these two sites, a new restriction site (BamHI) present within the linker itself was created at the corresponding

position. A second 20 bp linker was synthesized which connected the HindIII site from the LTR fragment to the PstI site from pGEM3Z, in this case without destruction of the recognition sequence on either of the ends and adding the three convenient unique restriction sites BglII, XhoI and EcoRV, to those already present in the polylinker of pGEM3Z, e.g. PstI, SalI, XhoI and BamHI. The resulting derivative of pGEM3Z, designated pVEC01, therefore contains a 650 bp restriction fragment carrying the LTR promoter sequence immediately followed by seven restriction sites available for the insertion of foreign genes. The 650 bp fragment is flanked on either end by a BamHI restriction site and has been transferred as such to the unique BglII site present in the 1,2 kb HVT insert from pMD07. The cohesive ends generated by these two restriction enzymes are compatible but ligation does not restore either of the original recognition sequences for BglII or BamHI. One of the resulting constructs, carrying the LTR in the orientation towards the TR_s, was designated pVECO4 and checked by restriction mapping (Figure 3). The structure of this universal HVT recombination vector allows the insertion of foreign genes immediately downstream of the LTR promoter and subsequent integration of the complete expression cassette into the HVT genome by in vivo recombination. The positions of the different restriction sites downstream of the LTR in particular those for the enzymes BglII, XhoI and EcoRV are designed in such a way that even multiple gene insertion can be envisaged. A 2,5 kb SalI/XhoI restriction fragment derived from pMD46 carrying the MD18 gene, was inserted into the unique BglII site of pVEC04 downstream of the LTR promoter, resulting in pMD48. A 3,6 kb SalI restriction fragment derived from pMD47 carrying the MD20 gene was inserted into the unique XhoI site of pVEC04 downstream of the LTR promoter, resulting in pMD49.

DNA of the plasmids pMD48 or pMD49 was introduced together with total DNA prepared from HVT infected cells into CEF by a method based on the calcium phosphate DNA precipitation according to Graham, F and v.d. Eb, A., (Virology 52, 456, 1973) with modifications described by Morgan et al. (Avian Diseases 34, 345, 1990). Two microgram of plasmid DNA from the constructs were mixed with 15 µg of DNA from HVT infected cells in a final volume of 560 µl H_2O and added to 750 µl of HBSP (20 mM KCl, 560 mM NaCl, 24 mM glucose, 3 mM Na_2HPO_4, 100 mM HEPES, pH 7,0). Precipitates were formed by gradually adding 190 µl of 1M $CaCl_2$ solution and incubating the mixtures at room-temperature for 30 minutes. In the meantime, 15 ml of a suspension of secondary CEF from 10 day old embryos in medium 6/B8, for which the composition is based on Glasgow's modification of Eagle's Minimal Essential Medium supplemented with 2% of foetal calf serum, were seeded in Ø 10 cm dishes at a density of 5×10^5 cells per ml. Calcium phosphate precipitated DNA was gently added to the cell suspension and dishes were incubated at 37 °C in a humified incubator containing 5% CO_2 in air. After 5 hours, medium was removed and 10 ml of solution containing equal volumes of HBSP and 30% glycerol was layered onto the cells. After a one to two minute incubation, the solution was removed, cells were washed with medium 6/B8 and dishes were incubated with fresh medium for 3 to 5 days until viral CPE developed. Detection of HVT recombinants expressing the MD18 or MD20 polypeptides was done by immunofluorescence staining using specific mono- or polyvalent sera against these MDV antigens.

Legends

Figure 1

Restriction enzyme map of a DNA fragment essentially corresponding to the Us region of the HVT genome. The relative position of the insertion-region consisting of four open reading frames and non-coding sequences in between is indicated.

Figure 2

A. Restriction enzyme map of pMD46, containing the gene encoding MD18-antigen flanked by SalI and XhoI restriction sites. Vector plasmid was derived from pSP72 (Promega, Wisconsin, U.S.A.) by modification of the ClaI restriction site in the polylinker into SalI.

B. Restriction enzyme map of pMD47, containing the gene encoding MD20-antigen flanked by SalI restriction sites. Vector plasmid was derived from pSP72 (Promega, Wisconsin, U.S.A.) by modification of the ClaI restriction site in the polylinker into SalI.

Figure 3

Restriction enzyme map of pVECO4 showing the LTR-promoter inserted into the unique BglII site of the 1,2 kb XhoI HVT fragment from pMD07.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: AKZO NV
(B) STREET: Velperweg 76
(C) CITY: Arnhem
(E) COUNTRY: the Netherlands
(F) POSTAL CODE (ZIP): 6824 BM

(ii) TITLE OF INVENTION: Recombinant Marek's disease virus

(iv) COMPUTER READABLE FORM:
Not Applicable

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2015 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Marek's disease herpesvirus
(B) STRAIN: GA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..2005
(D) OTHER INFORMATION: /label= pMD18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Gly Tyr Leu Ala Leu Tyr Gly Ala Asp Glu Ser Asp Glu Leu Asn Ile
         15                  20                  25

GAC CGC AAA GAT ATT CGC GCC GCG ATT CCG ACA CCA GCT CCT TTA CCA     145
Asp Arg Lys Asp Ile Arg Ala Ala Ile Pro Thr Pro Ala Pro Leu Pro
     30                  35                  40

ATA AAT ATA GAT CAC AGA AGA GAT TGC ACA GTC GGA GCA GTT CTT GCG     193
Ile Asn Ile Asp His Arg Arg Asp Cys Thr Val Gly Ala Val Leu Ala
 45                  50                  55                  60
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CTA ATA GAT GAT GAA CAT GGA TTA TTT TTC CTG GGA AAG ATA AAT TGT     241
Leu Ile Asp Asp Glu His Gly Leu Phe Phe Leu Gly Lys Ile Asn Cys
                  65                  70                  75

CCT GTG ATG GTA CGT ACA CTA GAG ACA GCC GCC AGT CAA GAA ATA TTC     289
Pro Val Met Val Arg Thr Leu Glu Thr Ala Ala Ser Gln Glu Ile Phe
              80                  85                  90

AGC GAA CTT GAT AAT CTT AAA CCA GAT GAT AAA TTG CTA TAT ATA ATT     337
Ser Glu Leu Asp Asn Leu Lys Pro Asp Asp Lys Leu Leu Tyr Ile Ile
          95                 100                 105

ACA AAT TAT CTT CCA TCG GTA TCG CTG TCC TCA CGA CGC CTA GCA CCG     385
Thr Asn Tyr Leu Pro Ser Val Ser Leu Ser Ser Arg Arg Leu Ala Pro
      110                 115                 120

GGG GAA ACG GCA GAT GAG ACT TTT TTG GCA CAT GTT GCT TTG TGT TTA     433
Gly Glu Thr Ala Asp Glu Thr Phe Leu Ala His Val Ala Leu Cys Leu
  125                 130                 135                 140

TTG GGG AAG CGA ATT GGA ACT ATT GTT ACA TAT GAT CTC ACC CCG GAA     481
Leu Gly Lys Arg Ile Gly Thr Ile Val Thr Tyr Asp Leu Thr Pro Glu
                  145                 150                 155

GAG GCT ATA GAG CCG TTC AGA AAG CTT TCT CCA AAT TCT AAA GCG ACC     529
Glu Ala Ile Glu Pro Phe Arg Lys Leu Ser Pro Asn Ser Lys Ala Thr
              160                 165                 170

TTG CTA TCA CAG GGC AAG GAA ACT GAA CGG CTC TTA GGT GAG ATG GTG     577
Leu Leu Ser Gln Gly Lys Glu Thr Glu Arg Leu Leu Gly Glu Met Val
          175                 180                 185

TGG TAT CCG AGC AAA AAT GCA ATA ACC AAA GCG TTA TTA GGA ACG GCG     625
Trp Tyr Pro Ser Lys Asn Ala Ile Thr Lys Ala Leu Leu Gly Thr Ala
      190                 195                 200

GTT AAT AAT ATG TTA CTG CGA GAT AGA TGG CAA ATT ATC TCC GAA CGA     673
Val Asn Asn Met Leu Leu Arg Asp Arg Trp Gln Ile Ile Ser Glu Arg
  205                 210                 215                 220

AGA CGC ATG GCT GGT ATA ACT GGA CAA AAG TAT TTG CAA GCA TCA TCT     721
Arg Arg Met Ala Gly Ile Thr Gly Gln Lys Tyr Leu Gln Ala Ser Ser
                  225                 230                 235

TTT ACG GCA TTG ACC GAT TCA ATG ACG TCA AAT AAC GTG TCA GTC ACC     769
Phe Thr Ala Leu Thr Asp Ser Met Thr Ser Asn Asn Val Ser Val Thr
              240                 245                 250

CAC CCA ATT TGT GAA AAC GCA AAC CCG GGT AAC ATA CAA AAG GAT GAG     817
His Pro Ile Cys Glu Asn Ala Asn Pro Gly Asn Ile Gln Lys Asp Glu
          255                 260                 265

GAA ATG CAA GTG TGT ATC AGT CCA GCA CAA ACG AGT GAA ACG TTA AAT     865
Glu Met Gln Val Cys Ile Ser Pro Ala Gln Thr Ser Glu Thr Leu Asn
      270                 275                 280
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GCT GGA GTG CTG TCT GGA TGC AAC GAT TTC CAT AGA CTT CCC CAC TCC    913
Ala Gly Val Leu Ser Gly Cys Asn Asp Phe His Arg Leu Pro His Ser
285                 290                 295                 300

GAC CCT GCA TCA ACG AGC GAT CAA ACC AAT TTG CAA TCG CTA ATA GAA    961
Asp Pro Ala Ser Thr Ser Asp Gln Thr Asn Leu Gln Ser Leu Ile Glu
                305                 310                 315

CCG TCC ATG AAC ACT CAA TCT TCT CGC CCA CCC GGA GAC GAT TTT ATT   1009
Pro Ser Met Asn Thr Gln Ser Ser Arg Pro Pro Gly Asp Asp Phe Ile
            320                 325                 330

TGG GTC CCG ATT AAA AGC TAT AAT CAG CTA GTA TCG AGA AAT GCT TCT   1057
Trp Val Pro Ile Lys Ser Tyr Asn Gln Leu Val Ser Arg Asn Ala Ser
        335                 340                 345

CAG CCA ACG AAT ATT CCC GAT ATT GCA ATT ACA TCG AAT CAG CCT CCG   1105
Gln Pro Thr Asn Ile Pro Asp Ile Ala Ile Thr Ser Asn Gln Pro Pro
    350                 355                 360

TTT ATT CCC CCG GCG TTA ATG AAT ACA TCG ATA TCA GGT CAA CAC TCC   1153
Phe Ile Pro Pro Ala Leu Met Asn Thr Ser Ile Ser Gly Gln His Ser
365                 370                 375                 380

ATC CCA AGT GGA TAT GCC CAA TAT GGG TAC CCT ACA CCC GTA GGT ACC   1201
Ile Pro Ser Gly Tyr Ala Gln Tyr Gly Tyr Pro Thr Pro Val Gly Thr
                385                 390                 395

CAT AAC TCT CTG CTT CCA TTG GGA CCT GTA AAT CAA ATG GGC GGA TTT   1249
His Asn Ser Leu Leu Pro Leu Gly Pro Val Asn Gln Met Gly Gly Phe
            400                 405                 410

CAA TAT GGA CCT CAG GTG TAC CCC TTG TCA TAT GGA CAA TCG CCT TTA   1297
Gln Tyr Gly Pro Gln Val Tyr Pro Leu Ser Tyr Gly Gln Ser Pro Leu
        415                 420                 425

GAA GCA AAA CTG ACA GCC TTA CTT GAA TGC ATG ACA AAG GAA AAG AGA   1345
Glu Ala Lys Leu Thr Ala Leu Leu Glu Cys Met Thr Lys Glu Lys Arg
    430                 435                 440

CCA GTG GAT GAG GAG CAC AGA GGC GAC GAT ATG CAT ACT ACT AGG GAA   1393
Pro Val Asp Glu Glu His Arg Gly Asp Asp Met His Thr Thr Arg Glu
445                 450                 455                 460

GAA CGA GGA CGA CGT GGA CGT AAG CGA CCA TAC GAA TTT GAC AGA TCT   1441
Glu Arg Gly Arg Arg Gly Arg Lys Arg Pro Tyr Glu Phe Asp Arg Ser
                465                 470                 475

ATC GAG TCT GAT CTT TAT TAT CCC GGT GAA TTC CGT CGG TCT AAT TTT   1489
Ile Glu Ser Asp Leu Tyr Tyr Pro Gly Glu Phe Arg Arg Ser Asn Phe
            480                 485                 490

TCT CCT CCT CAA GCC AGT AGT ATG AAA TAT GAA GAA ACT ACT GGG GGT   1537
Ser Pro Pro Gln Ala Ser Ser Met Lys Tyr Glu Glu Thr Thr Gly Gly
        495                 500                 505
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CGG CAT GAT CTG AGT CAA ACA GGA CCC GTA TTA AAT AGT CTA ATG GGA   1585
Arg His Asp Leu Ser Gln Thr Gly Pro Val Leu Asn Ser Leu Met Gly
    510                 515                 520

GCT GTG ACT TCC CTA CAA AAA GAA GTC GAA CGG CTA AAT GGA GGA AAT   1633
Ala Val Thr Ser Leu Gln Lys Glu Val Glu Arg Leu Asn Gly Gly Asn
525                 530                 535                 540

TTA CCG ATA TCA AAT GCA CAA AGT TCA TAT GGA GTG CCC AAT GGG ATG   1681
Leu Pro Ile Ser Asn Ala Gln Ser Ser Tyr Gly Val Pro Asn Gly Met
                545                 550                 555

CAT GCC CCA GTT TAT TAC TCA TAC CCT CCT CCG GGA ACA CAT CCC ACA   1729
His Ala Pro Val Tyr Tyr Ser Tyr Pro Pro Pro Gly Thr His Pro Thr
            560                 565                 570

GTT TCA TGG CCC ATG GGA GTC GAA CGC CCT ATG CCT TCC ACG GAA GGA   1777
Val Ser Trp Pro Met Gly Val Glu Arg Pro Met Pro Ser Thr Glu Gly
        575                 580                 585

AAA ACT TCT ACC AAT TCC ACG GTC ATT CCT GTG CCA GTT TCA GAT CCG   1825
Lys Thr Ser Thr Asn Ser Thr Val Ile Pro Val Pro Val Ser Asp Pro
    590                 595                 600

GAG GCT GGT CGA AAT GTA CCA ATA ACT GCG ACC ATC TCT CAG GAG CGT   1873
Glu Ala Gly Arg Asn Val Pro Ile Thr Ala Thr Ile Ser Gln Glu Arg
605                 610                 615                 620

TCC GAC GGA ATT CAG AAG GAA AGC ATC GAG CAA TCA CGG GAT ACC ATG   1921
Ser Asp Gly Ile Gln Lys Glu Ser Ile Glu Gln Ser Arg Asp Thr Met
                625                 630                 635

AAT GCT AGC GCC GTA GCT GGT ATA CAC CGC ACC AGT GAT GCC GGC GTC   1969
Asn Ala Ser Ala Val Ala Gly Ile His Arg Thr Ser Asp Ala Gly Val
            640                 645                 650

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 663 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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            20                  25                  30

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        35                  40                  45

His Arg Arg Asp Cys Thr Val Gly Ala Val Leu Ala Leu Ile Asp Asp
    50                  55                  60

Glu His Gly Leu Phe Phe Leu Gly Lys Ile Asn Cys Pro Val Met Val
65                  70                  75                  80

Arg Thr Leu Glu Thr Ala Ala Ser Gln Glu Ile Phe Ser Glu Leu Asp
                85                  90                  95

Asn Leu Lys Pro Asp Asp Lys Leu Leu Tyr Ile Ile Thr Asn Tyr Leu
            100                 105                 110

Pro Ser Val Ser Leu Ser Ser Arg Arg Leu Ala Pro Gly Glu Thr Ala
        115                 120                 125

Asp Glu Thr Phe Leu Ala His Val Ala Leu Cys Leu Leu Gly Lys Arg
    130                 135                 140

Ile Gly Thr Ile Val Thr Tyr Asp Leu Thr Pro Glu Glu Ala Ile Glu
145                 150                 155                 160

Pro Phe Arg Lys Leu Ser Pro Asn Ser Lys Ala Thr Leu Leu Ser Gln
                165                 170                 175

Gly Lys Glu Thr Glu Arg Leu Leu Gly Glu Met Val Trp Tyr Pro Ser
            180                 185                 190

Lys Asn Ala Ile Thr Lys Ala Leu Leu Gly Thr Ala Val Asn Asn Met
        195                 200                 205

Leu Leu Arg Asp Arg Trp Gln Ile Ile Ser Glu Arg Arg Arg Met Ala
    210                 215                 220

Gly Ile Thr Gly Gln Lys Tyr Leu Gln Ala Ser Ser Phe Thr Ala Leu
225                 230                 235                 240

Thr Asp Ser Met Thr Ser Asn Asn Val Ser Val Thr His Pro Ile Cys
                245                 250                 255

Glu Asn Ala Asn Pro Gly Asn Ile Gln Lys Asp Glu Glu Met Gln Val
            260                 265                 270

Cys Ile Ser Pro Ala Gln Thr Ser Glu Thr Leu Asn Ala Gly Val Leu
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Ser Gly Cys Asn Asp Phe His Arg Leu Pro His Ser Asp Pro Ala Ser
    290                 295                 300
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325 330 335

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420 425 430

Thr Ala Leu Leu Glu Cys Met Thr Lys Glu Lys Arg Pro Val Asp Glu
435 440 445

Glu His Arg Gly Asp Asp Met His Thr Thr Arg Glu Glu Arg Gly Arg
450 455 460

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465 470 475 480

Leu Tyr Tyr Pro Gly Glu Phe Arg Arg Ser Asn Phe Ser Pro Pro Gln
485 490 495

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500 505 510

Ser Gln Thr Gly Pro Val Leu Asn Ser Leu Met Gly Ala Val Thr Ser
515 520 525

Leu Gln Lys Glu Val Glu Arg Leu Asn Gly Gly Asn Leu Pro Ile Ser
530 535 540

Asn Ala Gln Ser Ser Tyr Gly Val Pro Asn Gly Met His Ala Pro Val
545 550 555 560

Tyr Tyr Ser Tyr Pro Pro Pro Gly Thr His Pro Thr Val Ser Trp Pro
565 570 575

Met Gly Val Glu Arg Pro Met Pro Ser Thr Glu Gly Lys Thr Ser Thr
580 585 590

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Asn Ser Thr Val Ile Pro Val Pro Val Ser Asp Pro Glu Ala Gly Arg
        595                 600                 605

Asn Val Pro Ile Thr Ala Thr Ile Ser Gln Glu Arg Ser Asp Gly Ile
    610                 615                 620

Gln Lys Glu Ser Ile Glu Gln Ser Arg Asp Thr Met Asn Ala Ser Ala
625                 630                 635                 640

Val Ala Gly Ile His Arg Thr Ser Asp Ala Gly Val Asp Val Phe Ile
                645                 650                 655

Asn Gln Met Met Ala His Gln
            660
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3265 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Marek's disease herpesvirus
(B) STRAIN: GA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 41..3265
(D) OTHER INFORMATION: /label= pMD20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Ser Ile Ser Asp Thr Leu Glu Ser Asp Asp Ser Gly Ile Lys Val Leu
                10                  15                  20

TTT GCC GTA GAT GGT TGT GCC GTG TCG TTT TCC CTG GCC CTT CTT ACA     151
Phe Ala Val Asp Gly Cys Ala Val Ser Phe Ser Leu Ala Leu Leu Thr
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Gly Gln Ile Pro Ser Thr Asn Ser Val Tyr Val Ile Gly Tyr Trp Asp
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Pro Ser Asp Arg Phe Ser Ser Ile Pro Phe Leu Asp Gly Asp Pro Asn
    55                  60                  65
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ACT AAT GAG AGA ATA TCT ACC ACC GTT TGT AAT TTA GAG GAT GTT CCC      295
Thr Asn Glu Arg Ile Ser Thr Thr Val Cys Asn Leu Glu Asp Val Pro
 70                  75                  80                  85

AGC CCT CTA AGA GTA GAA TTT TGT CTT CTG AAC CAA ATG GCA TCA GGT      343
Ser Pro Leu Arg Val Glu Phe Cys Leu Leu Asn Gln Met Ala Ser Gly
                 90                  95                 100

ATG GGC GGT GCT GAT TTA AAA CTG AGA ACA CGT GCA ATA TTC GTA TGC      391
Met Gly Gly Ala Asp Leu Lys Leu Arg Thr Arg Ala Ile Phe Val Cys
            105                 110                 115

CGA TTT ACA TCA TGG TCC GAA ATG AAC GCT ATC GCA AAT TCA ATA ATT      439
Arg Phe Thr Ser Trp Ser Glu Met Asn Ala Ile Ala Asn Ser Ile Ile
        120                 125                 130

TAT GGA ACG CCA ATT CAA GCC GGT GTT TTA CAA GCA ACA ATA TCT GAA      487
Tyr Gly Thr Pro Ile Gln Ala Gly Val Leu Gln Ala Thr Ile Ser Glu
    135                 140                 145

ACT GAA ACG TTC ATG TTA CAT GAT GAA TTC AAC CTT GCT CTT CAC GTC      535
Thr Glu Thr Phe Met Leu His Asp Glu Phe Asn Leu Ala Leu His Val
150                 155                 160                 165

TTT CTC AAT GGG TTA TCT CTG AAG GGT CGT AAC AAA AAA GAT GTT TGT      583
Phe Leu Asn Gly Leu Ser Leu Lys Gly Arg Asn Lys Lys Asp Val Cys
                170                 175                 180

ATG TCA TTG AAT CAC AAT TAT ATA TCG AGC GTA TCT GAG AAT TTC CCA      631
Met Ser Leu Asn His Asn Tyr Ile Ser Ser Val Ser Glu Asn Phe Pro
            185                 190                 195

AGG GGT AAA CGA GGT CTG ACT GGA CTC TAT TTA CAA CAC GAA CAA AAG      679
Arg Gly Lys Arg Gly Leu Thr Gly Leu Tyr Leu Gln His Glu Gln Lys
        200                 205                 210

GTC ACA GCA GCA TAT CGG CGT ATA TAT GGT GGA TCT ACT ACA ACT GCT      727
Val Thr Ala Ala Tyr Arg Arg Ile Tyr Gly Gly Ser Thr Thr Thr Ala
    215                 220                 225

TTT TGG TAC GTG TCC AAA TTC GGA CCA GAT GAA AAA AGT CTT GTT TTG      775
Phe Trp Tyr Val Ser Lys Phe Gly Pro Asp Glu Lys Ser Leu Val Leu
230                 235                 240                 245

GCC CTA CGT TAT TAC CTT TTG CAG GCA CAG GAA GAA GTT ACT GGT ATT      823
Ala Leu Arg Tyr Tyr Leu Leu Gln Ala Gln Glu Glu Val Thr Gly Ile
                250                 255                 260

GCA ACA GGC TAT GAT CTG CAA GCC ATA AAA GAT ATA TGC AAA ACA TAC      871
Ala Thr Gly Tyr Asp Leu Gln Ala Ile Lys Asp Ile Cys Lys Thr Tyr
            265                 270                 275

GCA GTG TCG GTA AAT CCC AAT CCC ACG GGA TTT TTG GCT GCC GAT TTA      919
Ala Val Ser Val Asn Pro Asn Pro Thr Gly Phe Leu Ala Ala Asp Leu
        280                 285                 290
```

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ACG TCA TTT AGT AGA TTA TCA CGT TTT TGT TGT TTA AGT TAC TAT TCC      967
Thr Ser Phe Ser Arg Leu Ser Arg Phe Cys Cys Leu Ser Tyr Tyr Ser
    295                 300                 305

AAA GGC TCT GTG GCC ATA GCA TTT CCA TCA TAT GTG GAA CGC AGG ATT     1015
Lys Gly Ser Val Ala Ile Ala Phe Pro Ser Tyr Val Glu Arg Arg Ile
310                 315                 320                 325

ATG GCC GAT ATC GCA GAA GTG GAT GCA TTG AGA GAA TAT ATA GAA AGA     1063
Met Ala Asp Ile Ala Glu Val Asp Ala Leu Arg Glu Tyr Ile Glu Arg
                330                 335                 340

GAC AGA CCC AGT TTG AAG ATT TCG GAT TTG GAA TTC GTT AAA TAT ATA     1111
Asp Arg Pro Ser Leu Lys Ile Ser Asp Leu Glu Phe Val Lys Tyr Ile
            345                 350                 355

TAT TTA GCT TAT TTT GAA TGT TAT AAC CGC GAA CAG TTA AAA CGA CAT     1159
Tyr Leu Ala Tyr Phe Glu Cys Tyr Asn Arg Glu Gln Leu Lys Arg His
        360                 365                 370

TTG AAA GAT GTG ACA GTA AGT TTG CCC GAT GAA GAC ATT TAC AAG AAG     1207
Leu Lys Asp Val Thr Val Ser Leu Pro Asp Glu Asp Ile Tyr Lys Lys
    375                 380                 385

TCT TCA CTA GGC AAG TGT GCA GTA GAA AAT TTT TTT ACA CAT GTG AGA     1255
Ser Ser Leu Gly Lys Cys Ala Val Glu Asn Phe Phe Thr His Val Arg
390                 395                 400                 405

TCT AGA TTG AAC GTG AAT GAC CAC ATA GCC CAT AAT GTA TTG CCC GAA     1303
Ser Arg Leu Asn Val Asn Asp His Ile Ala His Asn Val Leu Pro Glu
                410                 415                 420

CAA GTA GAA ATG GGA AAT AAG CTA GTC CGA AAG TTT GGA CGT GCC AGA     1351
Gln Val Glu Met Gly Asn Lys Leu Val Arg Lys Phe Gly Arg Ala Arg
            425                 430                 435

ATG TAT CTG TCA ACT ACG ATG ACT AAC GAG TCG CAC TTC ACT GGA ATA     1399
Met Tyr Leu Ser Thr Thr Met Thr Asn Glu Ser His Phe Thr Gly Ile
        440                 445                 450

TGT GAA TGT GCA TCT GTG ATT TTA AAG CGA CTG GAC ACT CTA GAA ATG     1447
Cys Glu Cys Ala Ser Val Ile Leu Lys Arg Leu Asp Thr Leu Glu Met
    455                 460                 465

AAA TTG CAA AAG TAT GGT TGG CCG TCT GAT CGT GTG GAT GGT TCC AAT     1495
Lys Leu Gln Lys Tyr Gly Trp Pro Ser Asp Arg Val Asp Gly Ser Asn
470                 475                 480                 485

CTA ATG GCC GAT AAT CAG AAC AAC TCT ACT TTA ATA CCG TAT GAT AAA     1543
Leu Met Ala Asp Asn Gln Asn Asn Ser Thr Leu Ile Pro Tyr Asp Lys
                490                 495                 500

TCT AGG TCT TCT GGA ATG ATA CTC GAG TGT TCG AAC ACT CAT TCT CGA     1591
Ser Arg Ser Ser Gly Met Ile Leu Glu Cys Ser Asn Thr His Ser Arg
            505                 510                 515
```

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GGG GGG CCG ATG ATA GTT AAA AGG TTA TTA GCT TTA GTA TCT GCC GAT   1639
Gly Gly Pro Met Ile Val Lys Arg Leu Leu Ala Leu Val Ser Ala Asp
        520                 525                 530

TCT CGC GCA GGG GGA ATC GGC CCA GCT AAC ATG CTC ATG GGG ATT GAC   1687
Ser Arg Ala Gly Gly Ile Gly Pro Ala Asn Met Leu Met Gly Ile Asp
    535                 540                 545

TCT GCA ATA GAT GGA CCC CTT CCA GTT TAC CGT GTG GGC ATG TCA AAG   1735
Ser Ala Ile Asp Gly Pro Leu Pro Val Tyr Arg Val Gly Met Ser Lys
550                 555                 560                 565

GGC AGA CAG GCT TTT ACG GTG CTT ATG ACC GAA TGT TGG GAA AGG ACC   1783
Gly Arg Gln Ala Phe Thr Val Leu Met Thr Glu Cys Trp Glu Arg Thr
                570                 575                 580

ATT CCA TCT CCG GGA AGT GCG AAA GCG CAT TTG ATC AAG CTT AAC AAC   1831
Ile Pro Ser Pro Gly Ser Ala Lys Ala His Leu Ile Lys Leu Asn Asn
            585                 590                 595

TCT TAC GGT ACT TCG ACA GAA GAC TTG ATT TCA CGA GAC TTA TTC CTA   1879
Ser Tyr Gly Thr Ser Thr Glu Asp Leu Ile Ser Arg Asp Leu Phe Leu
        600                 605                 610

ACT TCT GAA ATC GAA CAG CTT ATC GGA AGC ACA GTA GAA TTG CCG GAG   1927
Thr Ser Glu Ile Glu Gln Leu Ile Gly Ser Thr Val Glu Leu Pro Glu
    615                 620                 625

ATT ACA TGT GGC TCT GCC GAT GAA CAG CAA TAT ATA AAC CGC AAT GAA   1975
Ile Thr Cys Gly Ser Ala Asp Glu Gln Gln Tyr Ile Asn Arg Asn Glu
630                 635                 640                 645

GTC TTT AAT GGG AAT CTT GCG ATA GGA AAT ATA GTT TTA GAT GTG GAT   2023
Val Phe Asn Gly Asn Leu Ala Ile Gly Asn Ile Val Leu Asp Val Asp
                650                 655                 660

ATA CAT TTA AGA AAC CCC ATA CCT CTT AGA CTT ATG CAT GCA GCG ATA   2071
Ile His Leu Arg Asn Pro Ile Pro Leu Arg Leu Met His Ala Ala Ile
            665                 670                 675

CGA GGT TTT AGA AGT GGT ATA CTC AGA GCT TTG GCC TTA TTG CTA CCA   2119
Arg Gly Phe Arg Ser Gly Ile Leu Arg Ala Leu Ala Leu Leu Leu Pro
        680                 685                 690

AAG GCA AAT ATC GAC CAT GGC TCA TAC CCG TGT TAC TTT TAT AAG AGT   2167
Lys Ala Asn Ile Asp His Gly Ser Tyr Pro Cys Tyr Phe Tyr Lys Ser
    695                 700                 705

TCG TGC AAG AAA TCT AGA GTA ATG GGG GGA GCG CCT TGG ATG CTC CAT   2215
Ser Cys Lys Lys Ser Arg Val Met Gly Gly Ala Pro Trp Met Leu His
710                 715                 720                 725

GAT GCA GAA CTT GCC CCA GAT TAT TCG ATG TTT GAA AAT GCG GAG TTT   2263
Asp Ala Glu Leu Ala Pro Asp Tyr Ser Met Phe Glu Asn Ala Glu Phe
                730                 735                 740
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GAT TTA GAA ATG GGC ATA GAT GAC CCT TTA CTC ATA GAC CAA ATA GAT     2311
Asp Leu Glu Met Gly Ile Asp Asp Pro Leu Leu Ile Asp Gln Ile Asp
            745                 750                 755

GAA TCT CTT ACT AGA TGG AGC TCA GAA TCA TCA AGG AGT GTC GAT TTG     2359
Glu Ser Leu Thr Arg Trp Ser Ser Glu Ser Ser Arg Ser Val Asp Leu
        760                 765                 770

GAT CCA GAT AAG CCA TGC GGT TGC CAT GAT AAA ATC GGA TTG AGG GTT     2407
Asp Pro Asp Lys Pro Cys Gly Cys His Asp Lys Ile Gly Leu Arg Val
    775                 780                 785

TGC ATT CCA GTA CCC TCT CCA TAT TTA CTT GTG GGT AGC AAG ACA TTG     2455
Cys Ile Pro Val Pro Ser Pro Tyr Leu Leu Val Gly Ser Lys Thr Leu
790                 795                 800                 805

GCC GGA TTG TCT CGA ATC ATT CAA CAA GCC GTC CTC TTA GAG CGC AAT     2503
Ala Gly Leu Ser Arg Ile Ile Gln Gln Ala Val Leu Leu Glu Arg Asn
                810                 815                 820

TTT GTA GAA ACT ATA GGG CCA TAT CTG AAA AAT TAT GAG ATA ATT GAT     2551
Phe Val Glu Thr Ile Gly Pro Tyr Leu Lys Asn Tyr Glu Ile Ile Asp
            825                 830                 835

AGT GGC GTA TAT GGT CAT GGG CGT AGC TTA CGT CTG CCG TTT TTT GGC     2599
Ser Gly Val Tyr Gly His Gly Arg Ser Leu Arg Leu Pro Phe Phe Gly
        840                 845                 850

AAA ATT GAT GAA AAC GGT ATC GTG TCT AGA AGA CTT GTA CCG TTT TTC     2647
Lys Ile Asp Glu Asn Gly Ile Val Ser Arg Arg Leu Val Pro Phe Phe
    855                 860                 865

GTG ATA CCA GAT GAT TGT GCT GAC ATG GAG AAG TTT ATT GTG GCC CAT     2695
Val Ile Pro Asp Asp Cys Ala Asp Met Glu Lys Phe Ile Val Ala His
870                 875                 880                 885

TTC GAA CCT AAA AAC TTC CAT TTT CAC AGC TCT ATC CCG CTA GAA AAG     2743
Phe Glu Pro Lys Asn Phe His Phe His Ser Ser Ile Pro Leu Glu Lys
                890                 895                 900

GCC GCC ATA ATT CTG AAA GAT ATA GGT GGC GAA TAT GCA GGT TTC TTC     2791
Ala Ala Ile Ile Leu Lys Asp Ile Gly Gly Glu Tyr Ala Gly Phe Phe
            905                 910                 915

GAA AGA AAA ATT ACA GTA AAT AGA GAT ATA TTT TTC GGG ACT CGA TTA     2839
Glu Arg Lys Ile Thr Val Asn Arg Asp Ile Phe Phe Gly Thr Arg Leu
        920                 925                 930

TCT TTA TCA ATA GCT CTC AGG GAA AGG GGG GTA GAT ATA AAT GAT TGT     2887
Ser Leu Ser Ile Ala Leu Arg Glu Arg Gly Val Asp Ile Asn Asp Cys
    935                 940                 945

GCT GCC ATT ACA ACA TTT GTA ACA GAT CAC ATT TTA GAT GAT ATT ATA     2935
Ala Ala Ile Thr Thr Phe Val Thr Asp His Ile Leu Asp Asp Ile Ile
950                 955                 960                 965
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ACA TAC GTA TAT GAG CAT ATA CCA GAT CAC GCA ATC GAA TAT CAA AAT    2983
Thr Tyr Val Tyr Glu His Ile Pro Asp His Ala Ile Glu Tyr Gln Asn
                970                 975                 980

CTT TCT GTC TCG TGT TGT GTT GTC AAA TCG GAT TGG ATC CTG CTG CAG    3031
Leu Ser Val Ser Cys Cys Val Val Lys Ser Asp Trp Ile Leu Leu Gln
            985                 990                 995

CTA ATC CCC AAT AAA ACA ATA GGA TAT CGT CAC GGG TTT ACA TGT GTG    3079
Leu Ile Pro Asn Lys Thr Ile Gly Tyr Arg His Gly Phe Thr Cys Val
        1000                1005                1010

AGA TTT AAG CAT GCA AGA GCA AGG CGA GCG AGT GCA CGT TCT TAT TTG    3127
Arg Phe Lys His Ala Arg Ala Arg Arg Ala Ser Ala Arg Ser Tyr Leu
    1015                1020                1025

GCT CTG AAC GTC GAT GCG CAT GGT AGG TTG TGC GTA TGT GTA ATT CAA    3175
Ala Leu Asn Val Asp Ala His Gly Arg Leu Cys Val Cys Val Ile Gln
1030                1035                1040                1045

CAG TGT TTT GCG GCC AAG TGC GGA AAT AAT AAA CTT CGC ACA CTT TTC    3223
Gln Cys Phe Ala Ala Lys Cys Gly Asn Asn Lys Leu Arg Thr Leu Phe
                1050                1055                1060

ACG GTA GAT ATT GAC TCG AAA TGT CGA TTA GAA CAT CAA TAG             3265
Thr Val Asp Ile Asp Ser Lys Cys Arg Leu Glu His Gln
            1065                1070
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1074 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Arg Phe Ser Ser Ile Ser Asp Thr Leu Glu Ser Asp Asp Ser
 1               5                  10                  15

Gly Ile Lys Val Leu Phe Ala Val Asp Gly Cys Ala Val Ser Phe Ser
            20                  25                  30

Leu Ala Leu Leu Thr Gly Gln Ile Pro Ser Thr Asn Ser Val Tyr Val
        35                  40                  45

Ile Gly Tyr Trp Asp Pro Ser Asp Arg Phe Ser Ser Ile Pro Phe Leu
    50                  55                  60

Asp Gly Asp Pro Asn Thr Asn Glu Arg Ile Ser Thr Thr Val Cys Asn
65                  70                  75                  80
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Leu Glu Asp Val Pro Ser Pro Leu Arg Val Glu Phe Cys Leu Leu Asn
85 90 95

Gln Met Ala Ser Gly Met Gly Gly Ala Asp Leu Lys Leu Arg Thr Arg
100 105 110

Ala Ile Phe Val Cys Arg Phe Thr Ser Trp Ser Glu Met Asn Ala Ile
115 120 125

Ala Asn Ser Ile Ile Tyr Gly Thr Pro Ile Gln Ala Gly Val Leu Gln
130 135 140

Ala Thr Ile Ser Glu Thr Glu Thr Phe Met Leu His Asp Glu Phe Asn
145 150 155 160

Leu Ala Leu His Val Phe Leu Asn Gly Leu Ser Leu Lys Gly Arg Asn
165 170 175

Lys Lys Asp Val Cys Met Ser Leu Asn His Asn Tyr Ile Ser Ser Val
180 185 190

Ser Glu Asn Phe Pro Arg Gly Lys Arg Gly Leu Thr Gly Leu Tyr Leu
195 200 205

Gln His Glu Gln Lys Val Thr Ala Ala Tyr Arg Arg Ile Tyr Gly Gly
210 215 220

Ser Thr Thr Thr Ala Phe Trp Tyr Val Ser Lys Phe Gly Pro Asp Glu
225 230 235 240

Lys Ser Leu Val Leu Ala Leu Arg Tyr Tyr Leu Leu Gln Ala Gln Glu
245 250 255

Glu Val Thr Gly Ile Ala Thr Gly Tyr Asp Leu Gln Ala Ile Lys Asp
260 265 270

Ile Cys Lys Thr Tyr Ala Val Ser Val Asn Pro Asn Pro Thr Gly Phe
275 280 285

Leu Ala Ala Asp Leu Thr Ser Phe Ser Arg Leu Ser Arg Phe Cys Cys
290 295 300

Leu Ser Tyr Tyr Ser Lys Gly Ser Val Ala Ile Ala Phe Pro Ser Tyr
305 310 315 320

Val Glu Arg Arg Ile Met Ala Asp Ile Ala Glu Val Asp Ala Leu Arg
325 330 335

Glu Tyr Ile Glu Arg Asp Arg Pro Ser Leu Lys Ile Ser Asp Leu Glu
340 345 350

Phe Val Lys Tyr Ile Tyr Leu Ala Tyr Phe Glu Cys Tyr Asn Arg Glu
355 360 365

Gln Leu Lys Arg His Leu Lys Asp Val Thr Val Ser Leu Pro Asp Glu
370 375 380

Asp Ile Tyr Lys Lys Ser Ser Leu Gly Lys Cys Ala Val Glu Asn Phe
385 390 395 400

Phe Thr His Val Arg Ser Arg Leu Asn Val Asn Asp His Ile Ala His
405 410 415

Asn Val Leu Pro Glu Gln Val Glu Met Gly Asn Lys Leu Val Arg Lys
420 425 430

Phe Gly Arg Ala Arg Met Tyr Leu Ser Thr Thr Met Thr Asn Glu Ser
435 440 445

His Phe Thr Gly Ile Cys Glu Cys Ala Ser Val Ile Leu Lys Arg Leu
450 455 460

Asp Thr Leu Glu Met Lys Leu Gln Lys Tyr Gly Trp Pro Ser Asp Arg
465 470 475 480

Val Asp Gly Ser Asn Leu Met Ala Asp Asn Gln Asn Asn Ser Thr Leu
485 490 495

Ile Pro Tyr Asp Lys Ser Arg Ser Ser Gly Met Ile Leu Glu Cys Ser
500 505 510

Asn Thr His Ser Arg Gly Gly Pro Met Ile Val Lys Arg Leu Leu Ala
515 520 525

Leu Val Ser Ala Asp Ser Arg Ala Gly Gly Ile Gly Pro Ala Asn Met
530 535 540

Leu Met Gly Ile Asp Ser Ala Ile Asp Gly Pro Leu Pro Val Tyr Arg
545 550 555 560

Val Gly Met Ser Lys Gly Arg Gln Ala Phe Thr Val Leu Met Thr Glu
565 570 575

Cys Trp Glu Arg Thr Ile Pro Ser Pro Gly Ser Ala Lys Ala His Leu
580 585 590

Ile Lys Leu Asn Asn Ser Tyr Gly Thr Ser Thr Glu Asp Leu Ile Ser
595 600 605

Arg Asp Leu Phe Leu Thr Ser Glu Ile Glu Gln Leu Ile Gly Ser Thr
610 615 620

Val Glu Leu Pro Glu Ile Thr Cys Gly Ser Ala Asp Glu Gln Gln Tyr
625 630 635 640

Ile Asn Arg Asn Glu Val Phe Asn Gly Asn Leu Ala Ile Gly Asn Ile
645 650 655

Val Leu Asp Val Asp Ile His Leu Arg Asn Pro Ile Pro Leu Arg Leu
660 665 670

Met His Ala Ala Ile Arg Gly Phe Arg Ser Gly Ile Leu Arg Ala Leu
675 680 685

Ala Leu Leu Leu Pro Lys Ala Asn Ile Asp His Gly Ser Tyr Pro Cys
690 695 700

Tyr Phe Tyr Lys Ser Ser Cys Lys Lys Ser Arg Val Met Gly Gly Ala
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Pro Trp Met Leu His Asp Ala Glu Leu Ala Pro Asp Tyr Ser Met Phe
725 730 735

Glu Asn Ala Glu Phe Asp Leu Glu Met Gly Ile Asp Asp Pro Leu Leu
740 745 750

Ile Asp Gln Ile Asp Glu Ser Leu Thr Arg Trp Ser Ser Glu Ser Ser
755 760 765

Arg Ser Val Asp Leu Asp Pro Asp Lys Pro Cys Gly Cys His Asp Lys
770 775 780

Ile Gly Leu Arg Val Cys Ile Pro Val Pro Ser Pro Tyr Leu Leu Val
785 790 795 800

Gly Ser Lys Thr Leu Ala Gly Leu Ser Arg Ile Ile Gln Gln Ala Val
805 810 815

Leu Leu Glu Arg Asn Phe Val Glu Thr Ile Gly Pro Tyr Leu Lys Asn
820 825 830

Tyr Glu Ile Ile Asp Ser Gly Val Tyr Gly His Gly Arg Ser Leu Arg
835 840 845

Leu Pro Phe Phe Gly Lys Ile Asp Glu Asn Gly Ile Val Ser Arg Arg
850 855 860

Leu Val Pro Phe Phe Val Ile Pro Asp Asp Cys Ala Asp Met Glu Lys
865 870 875 880

Phe Ile Val Ala His Phe Glu Pro Lys Asn Phe His Phe His Ser Ser
885 890 895

Ile Pro Leu Glu Lys Ala Ala Ile Ile Leu Lys Asp Ile Gly Gly Glu
900 905 910

Tyr Ala Gly Phe Phe Glu Arg Lys Ile Thr Val Asn Arg Asp Ile Phe
915 920 925

Phe Gly Thr Arg Leu Ser Leu Ser Ile Ala Leu Arg Glu Arg Gly Val
930 935 940

Asp Ile Asn Asp Cys Ala Ala Ile Thr Thr Phe Val Thr Asp His Ile
945 950 955 960

Leu Asp Asp Ile Ile Thr Tyr Val Tyr Glu His Ile Pro Asp His Ala
965 970 975

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Ile Glu Tyr Gln Asn Leu Ser Val Ser Cys Cys Val Val Lys Ser Asp
            980                 985                 990

Trp Ile Leu Leu Gln Leu Ile Pro Asn Lys Thr Ile Gly Tyr Arg His
        995                1000                1005

Gly Phe Thr Cys Val Arg Phe Lys His Ala Arg Ala Arg Arg Ala Ser
   1010                1015                1020

Ala Arg Ser Tyr Leu Ala Leu Asn Val Asp Ala His Gly Arg Leu Cys
1025                1030                1035                1040

Val Cys Val Ile Gln Gln Cys Phe Ala Ala Lys Cys Gly Asn Asn Lys
                1045                1050                1055

Leu Arg Thr Leu Phe Thr Val Asp Ile Asp Ser Lys Cys Arg Leu Glu
            1060                1065                1070

His Gln
   1074
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Claims

1. A nucleic acid sequence encoding a MDV polypeptide characterized in that said sequence encodes at least a part of a polypeptide selected from the group consisting of:
   a MD18 having an amino acid sequence shown in SEQ ID NO:2
   b̄ MD20 having an amino acid sequence shown in SEQ ID NO:4
   and functional equivalents thereof.

2. A nucleic acid sequence according to claim 1, characterized in that said sequence at least contains a part of the deoxyribonucleic acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 3

3. A recombinant nucleic acid molecule comprising a nucleic acid sequence according to claims 1 or 2.

4. A recombinant nucleic acid molecule according to claim 3, characterized in that the nucleic acid sequence is operably linked to an expression control sequence.

5. A vector virus containing a nucleic acid sequence according to claims 1 or 2.

6. A host cell transformed with a nucleic acid sequence according to claims 1-2 or a recombinant nucleic acid molecule according to claims 3-4 or containing a vector virus according to claim 5.

7. A polypeptide displaying MDV immunological characteristics containing at least part of the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 4 or functional equivalents thereof.

8. A MDV polypeptide encoded by a nucleic acid sequence according to claims 1-2.

9. An antibody or antiserum immunoreactive with a polypeptide according to claims 7 or 8.

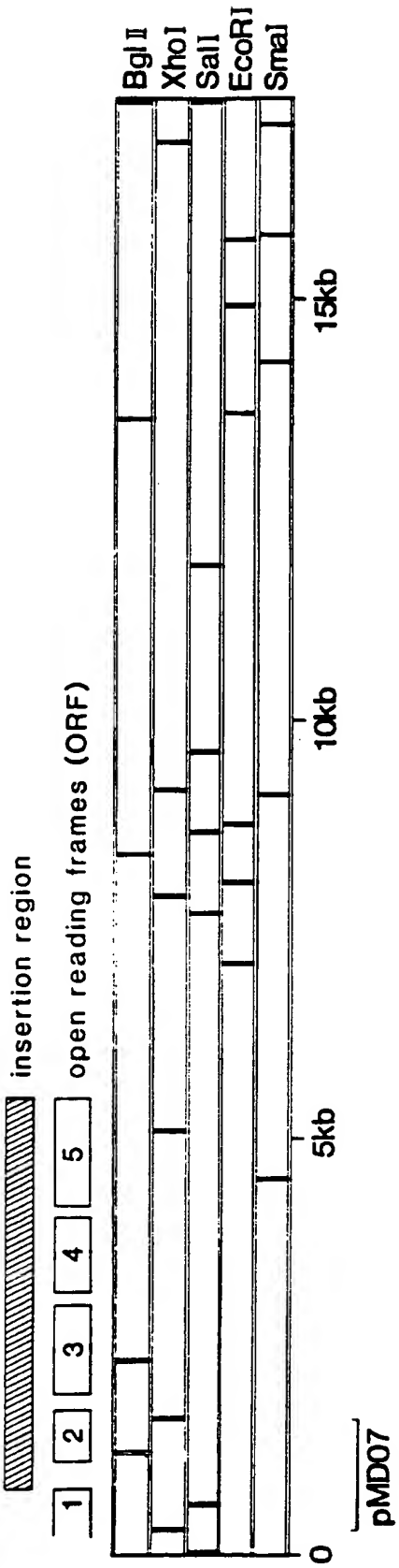
10. A vaccine for the protection of poultry against MDV infection, characterized in that it comprises a recombinant nucleic acid molecule according to claim 4, a vector virus according to claim 5, a host cell according to claim 6 or a polypeptide according to claims 7-8.

11. A method for preparation of MDV vaccine, characterized in that a host cell according to claim 6 is cultured, whereafter MDV containing material is collected and processed to a pharmaceutical preparation with immunizing activity.

12. A method for the preparation of an MDV vaccine, characterized in that a polypeptide according to claims 7-8 is processed to a pharmaceutical preparation with immunizing activity.

13. A method for the protection of poultry against MDV infection characterized in that an effective amount of a vaccine according to claim 10 is administered to the animals.

Fig 1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open reading frames (ORF)
1
2
3
4
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XhoI
SalI
EcoRI
SmaI
0
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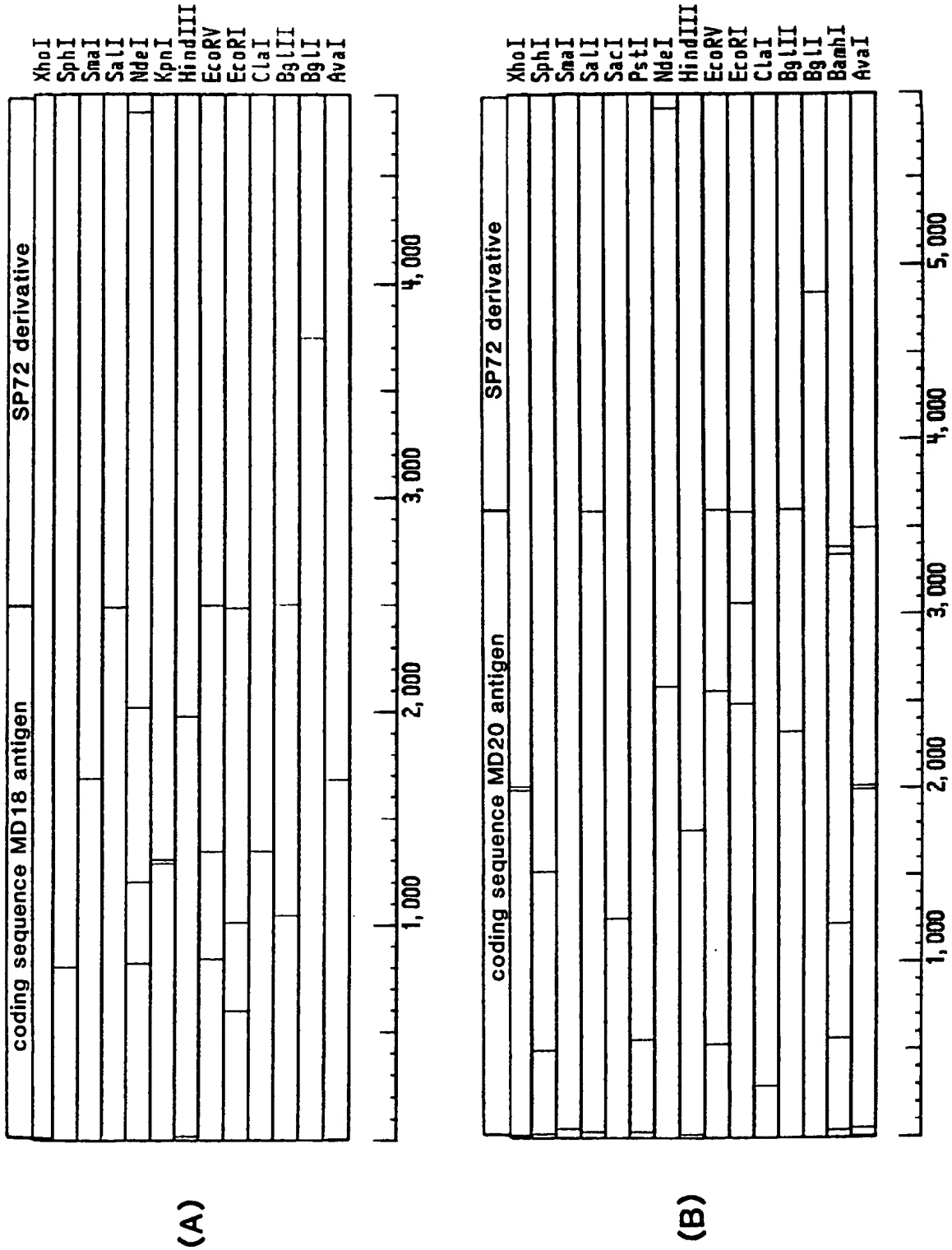
10kb
15kb
pMD07

Fig 2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SP72 derivative
XhoI
SphI
SmaI
SalI
NdeI
KpnI
HindIII
EcoRV
EcoRI
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BglI
AvaI
1,000
2,000
3,000
4,000
(A)
coding sequence MD20 antigen
SP72 derivative
XhoI
SphI
SmaI
SalI
SacI
PstI
NdeI
HindIII
EcoRV
EcoRI
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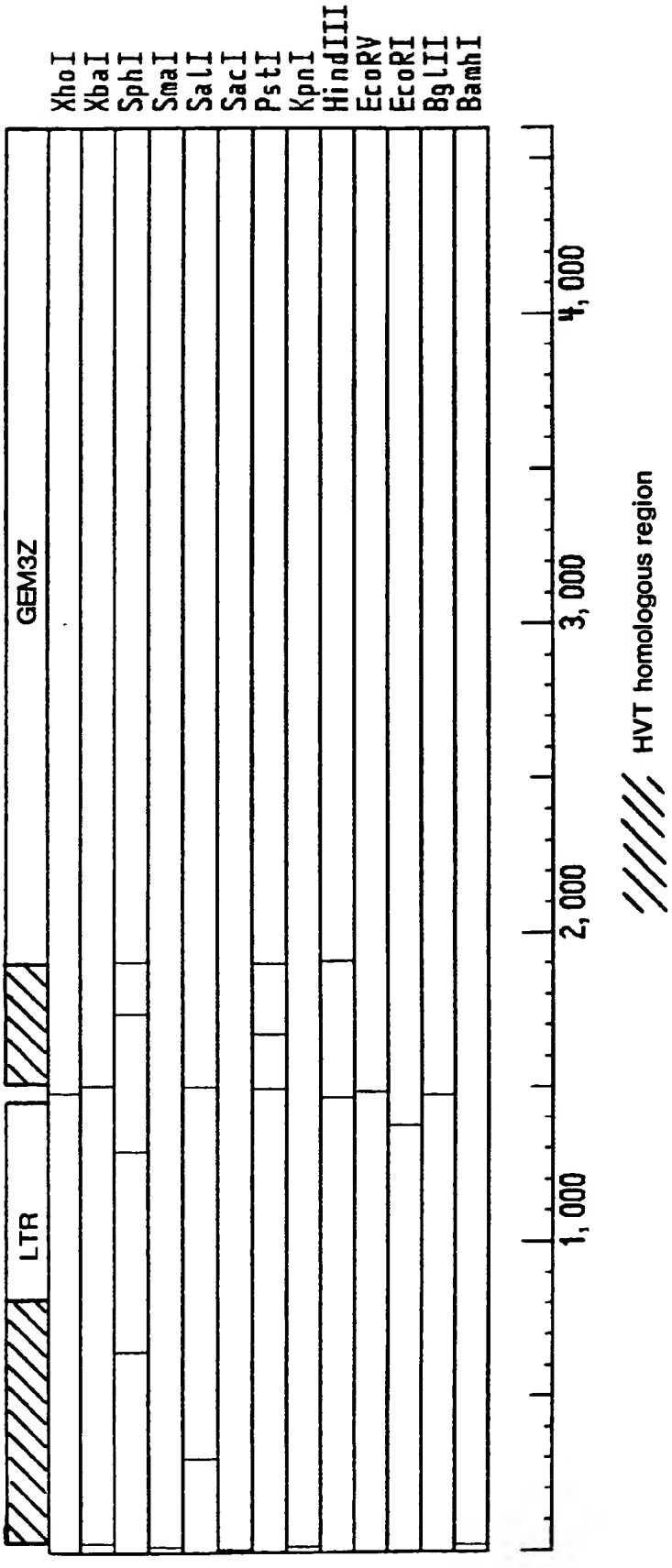
2,000
3,000
4,000
5,000
(B)

Fig 3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XbaI
SphI
SmaI
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KpnI
HindIII
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EcoRI
BglII
BamhI
GEM3Z
LTR
1,000
2,000
3,000
4,000
HVT homologous region

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European Patent Office
Office européen des brevets

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(54) **Marek's disease virus vaccine.**

(57) The invention is concerned with the MD18 and MD20 polypeptides of Marek's Disease virus which can be used to vaccinate poultry against MD.

The invention also relates to nucleic acid sequences encoding the MD18 or MD20 polypeptides. Said sequences can be used for the preparation of a subunit or vector vaccine.

EP 0 486 106 A3

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 91 20 2947

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
A	WO-A-9 002 803 (RHONE-MERIEUX) 22 March 1990 * the whole document * ---	1-13	C12N15/38 C12N15/86 C12N5/10 A61K39/255
A	J.GEN.VIROL. vol. 69, 1988, pages 2033 - 2042 BUCKMASTER, A.E., ET AL. 'Gene sequence and mapping data from Marek's Disease Virus and Herpesvirus of Turkeys: implications for herpevirus classification' * the whole document * ---	1-13	A61K39/395 C12P21/00
A	J.GEN.VIROL. vol. 69, 1988, pages 1531 - 1574 MCGOECH, D.J., ET AL. 'The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1' * page 1561 UL26 * --- -/--	1-13	

TECHNICAL FIELDS SEARCHED (Int. Cl. 5)

C12N
A61K
C12P

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	15 OCTOBER 1992	MADDOX A.D.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	BIOLOGICAL ABSTRACTS BR35:65580 * abstract * & J. AM. VET. MED. ASSOC.;125TH ANNUAL AMERICAN VETERINARY MEDICAL ASSOCIATION MEETING, JULY 17-26, 1988. vol. 192, no. 12, 1988, page 1789 CUI, Z., ET AL. 'Marek's disease virus gene clones constructed in bacteriophage and identified with monoclonal antibodies'	9	
A	J.IMMUNOL. vol. 130, no. 2, February 1983, pages 1003 - 1006 LEE, L.F., ET AL. 'Monoclonal antibodies with specificity for three different serotypes of Marek's Disease Virus in chickens' * the whole document *	9	TECHNICAL FIELDS SEARCHED (Int. Cl. 5)

Remark: Although claim 13 is directed to a method of treatment of the animal body (Art. 52(4) EPC) the search has been carried out and based on the alleged effects of the compound/composition.